

The invention claimed is:

1. A chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture, said method comprising:

(a) applying the mixture to an anion-exchange solid,

5 (b) eluting the solid of step (a) with a mobile phase comprising an eluting salt, an organic solvent, and a buffer, wherein said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and wherein the eluting results in the separation of said heteroduplexes from said homoduplexes.

10 2. A method of claim 1, wherein step (b) includes contacting the solid of step (a) with a mobile phase possessing a pH in the range of 4 to 9, said mobile phase comprising:

an eluting salt composed of equal concentrations of:

a cation selected from the group consisting of dialkylammonium,

15 trialkylammonium and tetraalkylammonium, or mixtures thereof, wherein the alkyl groups consist of any combination of methyl and ethyl; and

an anion selected from the group consisting of bromide, chloride, acetate, formate, nitrate, perchlorate, dihydrogen phosphate, ethane sulfonate, and methane sulfonate, or mixtures thereof;

20 a buffer acid with a pKa in the approximate range of 3.5 to 9.5; and, an organic solvent;

wherein the concentration of eluting salt is systematically increased from approximately 0.5M to approximately 2.0M.

25 3. A method of claim 2 wherein the eluting salt is systematically increased from approximately 1.0M to approximately 2.0M.

4. A method of claim 2 wherein said cation is selected from the group consisting of dialkylammonium, trialkylammonium and tetraalkylammonium wherein the alkyl groups consist of any combination of methyl and ethyl.

5. A method of claim 2 wherein said cation comprises choline.

30 6. A method of claim 2 wherein said cation comprises guanidinium.

7. A method of claim 2 wherein said cation comprises sodium.

8. A method of claim 2 wherein said anion is formate or chloride.

9. A method of claim 2 wherein said mobile phase includes a metal chelating agent.

5 10. A method of claim 9 wherein said metal chelating agent is selected from the group consisting of acetylacetone, alizarin, aluminon, chloranilic acid, kojic acid, morin, rhodizonic acid, thionalide, thiourea, α -furildioxime, nioxime, salicylaldoxime, dimethylglyoxime, α -furildioxime, cupferron, α -nitroso- β -naphthol, nitroso-R-salt, diphenylthiocarbazone, diphenylcarbazone,

10 eriochrome black T, PAN, SPADNS, glyoxal-bis(2-hydroxyanil), murexide, α -benzoinoxime, mandelic acid, anthranilic acid, ethylenediamine, glycine, triaminotriethylamine, thionalide, triethylenetetramine, EDTA, metalphthalein, arsonic acids, α , α '-bipyridine, 4-hydroxybenzothiazole, β -hydroxyquinaldine, β -hydroxyquinoline, 1,10-phenanthroline, picolinic acid, quinaldic acid, α , α ', 15 α "-terpyridyl, 9-methyl-2,3,7-trihydroxy-6-fluorone, pyrocatechol, rhodizonic acid, salicylaldoxime, salicylic acid, tiron, 4-chloro-1,2-dimercaptobenzene, dithiol, mercaptobenzothiazole, rubanic acid, oxalic acid, sodium diethyldithiocarbarbamate, zinc dibenzylidithiocarbamate, deferoxamine mesylate, crown ethers, and mixtures of any one or more of the above.

20 11. A method of claim 1, wherein said solid is comprised of a silica, polysaccharide or synthetic polyolefin backbone.

12. A method of claim 11 wherein said polyolefin is a polystyrene or polyacrylic.

25 13. A method of claim 1, wherein said solid comprises a polyacrylic backbone.

14. A method of claim 1, wherein said solid comprises diethylaminoethyl functional groups.

15. A method of claim 1, wherein said solid comprises polyethyleneimine functional groups.

30 16. A method of claim 1, wherein said solid comprises particles with an average diameter between approximately 2 micron and 10 micron.

17. A method of claim 1, wherein said solid is substantially nonporous.

18. A method of claim 1, wherein said solid comprises a polystyrene backbone.

19. A method of claim 1 wherein said mobile phase contains an organic solvent selected from the group consisting of methanol, ethanol, acetonitrile, ethyl acetate, formamide, 2-propanol, and N-methyl pyrrolidone.

20. A method of claim 1 wherein said mobile phase contains less than about 40% by volume of said organic solvent.

21. A method of claim 1 wherein said eluting is carried out at a column temperature greater than about 50°C.

22. A method of claim 1 wherein said eluting is carried out at a column temperature between about 40°C and about 80°C.

23. A method of claim 1 wherein the concentration of said eluting salt is continuously increased.

24. A method of claim 1 including analyzing the mobile phase after the elution step (b) for the concentration of said DNA molecules.

25. A method of claim 24 wherein the concentration of said DNA molecules is measured by ultraviolet absorbance in the approximate wavelength range of about 250 nm to about 290 nm.

26. A method of claim 1 wherein the total time required to complete said method is between about 2 minutes and about 30 minutes.

27. A method of claim 1 wherein the concentration of organic solvent is systematically increased.

28. A method of claim 1 where said solid is contained in a column of cylindrical geometry.

29. A chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture, comprising:

30. (a) applying the mixture to an anion-exchange solid,

(b) eluting the solid of step (a) with a mobile phase containing an eluting salt and a buffer, where said eluting is carried out under conditions

effective to at least partially denature said heteroduplexes and where the eluting results in the separation of said heteroduplexes from said homoduplexes.

30. A method of claim 29, wherein step (b) includes contacting the 5 solid of step (a) with a mobile phase possessing a pH in the range of 4 to 9 comprising:

an eluting salt composed of equal concentrations of:

a cation selected from the group consisting of dialkylammonium, trialkylammonium and tetraalkylammonium wherein the alkyl groups consist of 10 any combination of methyl and ethyl; and

an anion selected from the group consisting of bromide, chloride, acetate, formate, nitrate, perchlorate, dihydrogen phosphate, ethane sulfonate, and methane sulfonate; and

a buffer acid with a pKa in the approximate range of 3.5 to 9.5;

15 wherein the concentration of eluting salt is systematically increased from approximately 0.5M to approximately 2.0M.

31. A method of claim 30 wherein said cation is selected from the group consisting of dialkylammonium, trialkylammonium and tetraalkylammonium wherein the alkyl groups consist of any combination of 20 methyl and ethyl.

32. A method of claim 30 wherein said anion is selected from the group consisting of a bromide, chloride, acetate, formate, nitrate, perchlorate, dihydrogen phosphate, ethane sulfonate, and methane sulfonate.

33. A method of claim 30 wherein said cation comprises choline.

25 34. A method of claim 30 wherein said cation comprises sodium.

35. A method of claim 30 wherein said mobile phase includes a metal chelating agent.

36. A method of claim 35 wherein said metal chelating agent is 30 selected from the group consisting of acetylacetone, alizarin, aluminon, chloranilic acid, kojic acid, morin, rhodizonic acid, thionalide, thiourea, α -

furildioxime, nioxime, salicyaldoxime, dimethylglyoxime, α -furildioxime, cupferron, α -nitroso- β -naphthol, nitroso-R-salt, diphenylthiocarbazone, diphenylcarbazone, eriochrome black T, PAN, SPADNS, glyoxal-bis(2-hydroxyanil), murexide, α -benzoinoxime, mandelic acid, anthranilic acid,

5 ethylenediamine, glycine, triaminotriethylamine, thionalide, triethylenetetramine, EDTA, metalphthalein, arsonic acids, α, α' -bipyridine, 4-hydroxybenzothiazole, β -hydroxyquinaldine, β -hydroxyquinoline, 1,10-phenanthroline, picolinic acid, quinaldic acid, $\alpha, \alpha', \alpha''$ -terpyridyl, 9-methyl-2,3,7-trihydroxy-6-fluorone, pyrocatechol, rhodizonic acid, salicyaldoxime,

10 salicylic acid, tiron, 4-chloro-1,2-dimercaptobenzene, dithiol, mercaptobenzothiazole, rubanic acid, oxalic acid, sodium diethyldithiocarbarbamate, zinc dibenzylidithiocarbamate, deferoxamine mesylate, crown ethers, and mixtures of any one or more of the above.

37. A method of claim 30 wherein said cation comprises guanidinium.

15 38. A method of claim 30 wherein said anion is formate or chloride.

39. A method of claim 30 wherein the eluting salt is systematically increased from approximately 1.0M to approximately 2.0M.

40. A method of claim 30 including analyzing the mobile phase eluting from the column for the presence of DNA.

20 41. A method of claim 30 wherein said eluting is carried out at a column temperature greater than about 50°C.

42. A method of claim 30 wherein said eluting is carried out at a column temperature between about 40°C and about 80°C.

25 43. An aqueous mobile phase useful for the anion-exchange chromatography of double-stranded nucleic acids, said mobile phase comprising:

an eluting salt in the approximate concentration range of 0.5 to 1.5 M composed of equal concentrations of:

30 a cation selected from the group consisting of dialkylammonium, trialkylammonium and tetraalkylammonium wherein the alkyl groups consist of any combination of methyl and ethyl; and

an anion selected from the group consisting of bromide, chloride, acetate, formate, nitrate, perchlorate, dihydrogen phosphate, ethane sulfonate, and methane sulfonate; and

5 a buffer acid with a pKa in the approximate range of 3.5 to 9.5, which acid has a concentration not exceeding approximately 0.5M, and

an organic solvent, which organic solvent has a concentration not exceeding about 40% by volume of said mobile phase.

44. An aqueous mobile phase useful for the anion-exchange chromatography of double-stranded nucleic acids, said mobile phase

10 comprising:

an eluting salt in the approximate concentration range of 1.0M to 2.0M composed of equal concentrations of:

a cation selected from the group consisting of dialkylammonium, trialkylammonium and tetraalkylammonium wherein the alkyl groups consist of any combination of methyl and ethyl; and

15 an anion selected from the group consisting of bromide, chloride, acetate, formate, nitrate, perchlorate, dihydrogen phosphate, ethane sulfonate, and methane sulfonate; and

a buffer acid with a pKa in the approximate range of 3.5 to 9.5, which acid has a concentration not exceeding approximately 0.5M;

20 an organic solvent, which organic solvent has a concentration not exceeding about 40% by volume of said mobile phase.

45. A mobile phase of claim 43 wherein said cation is selected from the group consisting of dialkylammonium, trialkylammonium and

25 tetraalkylammonium wherein the alkyl groups consist of any combination of methyl and ethyl.

46. A mobile phase of claim 43 wherein said anion is formate or chloride.

47. A mobile phase of claim 43 wherein said cation comprises choline.

30 48. A mobile phase of claim 43 wherein said cation comprises sodium.

49. A mobile phase of claim 43 wherein said mobile phase includes a metal chelating agent.

50. A mobile phase of claim 49 wherein said metal chelating agent is selected from the group consisting of acetylacetone, alizarin, aluminon,

5 chloranilic acid, kojic acid, morin, rhodizonic acid, thionalide, thiourea, α -furildioxime, nioxime, salicylaldoxime, dimethylglyoxime, α -furildioxime, cupferron, α -nitroso- β -naphthol, nitroso-R-salt, diphenylthiocarbazone, diphenylcarbazone, eriochrome black T, PAN, SPADNS, glyoxal-bis(2-hydroxyanil), murexide, α -benzoinoxime, mandelic acid, anthranilic acid,

10 ethylenediamine, glycine, triaminotriethylamine, thionalide, triethylenetetramine, EDTA, metalphthalein, arsonic acids, α , α '-bipyridine, 4-hydroxybenzothiazole, β -hydroxyquinaldine, β -hydroxyquinoline, 1,10-phenanthroline, picolinic acid, quinaldic acid, α , α ', α "-terpyridyl, 9-methyl-2,3,7-trihydroxy-6-fluorone, pyrocatechol, rhodizonic acid, salicylaldoxime,

15 salicylic acid, tiron, 4-chloro-1,2-dimercaptobenzene, dithiol, mercaptobenzothiazole, rubeanic acid, oxalic acid, sodium diethyldithiocarbarbamate, zinc dibenzylidithiocarbamate, deferoxamine mesylate, crown ethers, and mixtures of any one or more of the above.

51. A mobile phase of claim 43 wherein said cation comprises

20 guanidinium.

52. A composition comprising the combination of the mobile phase of claim 43 with an anion-exchange solid.

53. A composition of claim 52 wherein said solid is comprised of a silica, polysaccharide or synthetic polyolefin backbone.

25 54. A composition of claim 52 wherein said polyolefin is a polystyrene or polyacrylic.

55. A composition of claim 52 wherein said mobile phase contains an organic solvent selected from the group consisting of methanol, ethanol, acetonitrile, ethyl acetate, formamide, 2-propanol, and N-methyl pyrrolidone.

30 56. A composition of claim 55 wherein said mobile phase contains less than about 40% by volume of said organic solvent.

57. A composition of claim 53 wherein said solid comprises diethylaminoethyl functional groups.

58. A composition of claim 53 wherein said solid comprises polyethyleneimine functional groups.

5 59. A composition of claim 52 wherein said solid comprises particles with an average diameter between approximately 2 micron and 10 micron.

60. A composition of claim 52 wherein said composition is retained at a temperature greater than about 30°C.

10 61. A composition of claim 52 wherein said composition is retained at a temperature greater than about 50°C.

62. A composition of claim 55 wherein said composition is retained at a temperature greater than about 60°C.

63. A composition of claim 52 wherein said composition is retained at a temperature greater than about 80°C.

15 64. A chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture, said method comprising:

(a) applying the mixture to an anion-exchange solid,

(b) eluting the solid of step (a) with a mobile phase containing an eluting salt, an organic solvent, and a buffer, wherein said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and wherein the eluting results in the separation of said heteroduplexes from said homoduplexes;

wherein step (b) includes contacting the solid of step (a) with a mobile phase possessing a pH in the range of 4 to 9 comprising:

25 an eluting salt comprising equal concentrations of:

a cation;

an anion;

a buffer acid with a pKa in the approximate range of 3.5 to 9.5; and,

an organic solvent;

wherein said mobile phase contains less than about 40% by volume of said organic solvent;

wherein the concentration of eluting salt is systematically increased from approximately 0.5M to approximately 2.0M.

5 65. A method of claim 63 wherein the eluting is carried out at a column temperature greater than about 50°C.

66. A chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture, comprising:

(a) applying the mixture to an anion-exchange solid,

10 (b) eluting the solid of step (a) with a mobile phase containing an eluting salt, an organic solvent, and a buffer, wherein said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and wherein the eluting results in the separation of said heteroduplexes from said homoduplexes;

15 wherein step (b) includes contacting the solid of step (a) with a mobile phase possessing a pH in the range of 4 to 9 comprising:

an eluting salt comprising:

betaine at a concentration in the range of about 0.5M to about 6M;

20 a buffer acid with a pKa in the approximate range of 3.5 to 9.5; and, an organic solvent;

wherein said mobile phase contains less than about 40% by volume of said organic solvent;

25 wherein the concentration of eluting salt is systematically increased from approximately 0.5M to approximately 2.0M.

67. A method of claim 66 wherein the eluting is carried out at a column temperature greater than about 50°C.

68. A chromatographic method for separating heteroduplex and homoduplex DNA molecules in a mixture, said method comprising:

30 (a) applying the mixture to an anion-exchange solid,

(b) eluting the solid of step (a) with a mobile phase containing an eluting salt, an organic solvent, and a buffer, where said eluting is carried out under conditions effective to at least partially denature said heteroduplexes and where the eluting results in the separation of said heteroduplexes from

5 said homoduplexes;

wherein step (b) includes contacting the solid of step (a) with a mobile phase possessing a pH in the range of 4 to 9 comprising:

an eluting salt comprising equal concentrations of:

a cation;

10 an anion;

a buffer acid with a pKa in the approximate range of 3.5 to 9.5; and,

wherein the eluting is carried out at a column temperature greater than about 50°C,

wherein the concentration of eluting salt is systematically increased
15 from approximately 0.5M to approximately 2.0M.

69. A method for detecting DNA genetic mutations, the method comprising:

a) heating a mixture of a sample double stranded DNA segment and a corresponding wild type double stranded DNA segment to a

20 temperature at which the strands are completely denatured;

b) cooling the product of step (a) until the strands are completely annealed, whereby a mixture comprising two homoduplexes and two heteroduplexes is formed if the sample segment includes a mutation;

c) determining the heteromutant site separation temperature;

25 d) analyzing the product of step (b) with Denaturing Anion-Exchange High Performance Chromatography at the heteromutant site separation temperature to identify the presence of any heteromutant site separated components therein.

70. A Method of Claim 69 wherein the heteromutant site separation

30 temperature is determined by analyzing the product of step (b) by Denaturing

65 Anion-Exchange High Performance Liquid Chromatography in a series of
66 incremental Denaturing Anion-Exchange High Performance Liquid

67 Chromatography separations in the mutation separation temperature range,
68 each successive separation having a higher temperature than the preceding

69 5 separation until a mutation separation profile is observed or the absence of
70 any mutation separation profile in the mutation separation temperature range
71 is observed, wherein a mutation separation profile identifies the presence of a
72 mutation and the absence of a mutation separation profile indicates an
73 absence of mutation in the sample.

74 10 71. A Method of Claim 69 wherein the heteromutant site separation
75 temperature is determined by analyzing the product of step (b) by Denaturing
76 Anion-Exchange High Performance Liquid Chromatography in a series of
77 incremental Denaturing Anion-Exchange High Performance Liquid

78 Chromatography separations in the mutation separation temperature range,
79 each successive separation having a lower temperature than the preceding

80 15 separation until a mutation separation profile is observed or the absence of
81 any mutation separation profile in the mutation separation temperature range
82 is observed, wherein a mutation separation profile identifies the presence of a
83 mutation and the absence of a mutation separation profile indicates an
84 absence of mutation in the sample.

85 20 72. The method of claim 1, where prior to said applying step the
86 DNA molecules are amplified using the polymerase chain reaction and the
87 amplified DNA molecules are denatured and renatured to form a mixture of
88 heteroduplex and homoduplex DNA molecules.